NCI ETI Branch
Flow Cytometry Core Laboratory

Scheduling multilaser analysis and cell sorting.

Multilaser analysis and cell sorting require the assistance of a trained operator. Contact Veena Kapoor at 5-6378 to make an appointment (if Veena cannot be reached, call Bill Telford at 5-6379). The cell sorting schedule fills up quickly - we strongly recommend that you make your appointment ***no less than one week in advance***. Although we will do our best to accomodate short-notice "emergencies", we cannot guarantee that time will be available with less than one week notice. If you need to cancel, please try to do so as early as possible.

You should meet with the operator several days prior to the sort to plan your experiment. Information we need includes...

- **What you are analyzing/sorting?** The type of cell you are analyzing and the phenotypes you are attempting to detect will dictate the choice of fluorochromes used - we can advise you on the best combinations. We will need to know the laser excitation wavelengths you will require (do you need UV, violet, red, etc. excitation sources?) as well as detection requirements (how many colors, what dichroics and filters, etc.). Go to the laser and filter/detector pages to see your options.

- **Number of cells required, time limitations and yield versus purity issues.** For sorting experiments we need to know how many cells you can provide, the estimated abundance of the cell type(s) of interest, and how many cells you need to recover at the end. The FACSVantage SE can sort at rates ranging from 200 to 20,000 cells/second, with yield and purity decreasing somewhat with increased sort rate. The fastest sort rate may therefore not be the best choice for your application. We can adjust the instrument to maximize either cell yield (with lower purity) or purity (with lower cell yield). Decisions need to be made in advance regarding the most optimal sort conditions for your particular experiment. Cell yields on the FACSVantage range from 50% to 80% recovery efficiency, depending on the sorting conditions. We can therefore give you estimates of the number of cells you can expect from your starting population, as well as the duration of the sort. Go to the sorting page to see our current performance levels.

- **Physical characteristics of the cells.** The physical characteristics of the cell type influence the sort conditions. Smaller or larger cell types may sort more efficiently through smaller or larger nozzles. Fragile cells (such as dendritic cells) or cells in a fragile state (including apoptotic cells) may sort more successfully through a larger nozzle at lower instrument

pressures and lower sort rates. Adherent cells may require a larger nozzle, as well as additions to the sample buffer to reduce clumping and prevent nozzle clogs. We have 50, 70, 80, 90, 100, 200, 300 and 400 µm diameter nozzles to accomodate most particle sizes.

- **Special instrument and equipment requirements.** We need to know in advance if you require sterile sorting conditions, since this requires overnight instrument sterilization prior to the sort. Some experiments require special equipment, such as the Cytek kinetic module for calcium flux measurement or the CloneCyt automatic cell deposition unit for automated cell plating and Index Sorting. These instrument preparations and modifications are time-consuming to set up and should be arranged well in advance. Retuning lasers to obtain unusual laser lines also needs to scheduled well in advance to avoid interfering with other users experiments.

Careful advance planning will minimize the time required for sorting and will reduce procedural and experimental errors!

Protocol for non-sterile cell sorting.

We generally adhere to the following conventions for cell sorting. Your sort will go far more quickly and efficiently if you read these instructions beforehand and ***arrive prepared***! Although we stock many of the supplies listed below, hood and bench space in the flow lab are currently non-existent - cell filtration, preparation of sort collection tubes, etc. should therefore be carried out in your own lab prior to sorting.

- We provide sheath buffer for non-sterile sorts. For fixed cell sorts (non-viable), we use FACFlow sheath buffer containing preservatives. For non-sterile live cell sorts we use Dulbecco's PBS as a sheath buffer. If you would like to provide your own sheath buffer, please deliver it to the flow lab the day before the sort.

- Viable cells should be resuspended in a low protein buffer for sorting - high protein concentrations can disrupt sort stream formation. We typically use 2% FBS in colorless HBSS, although any variation on this (BSA, RPMI, PBS) will generally work. Protein additions and media should be sufficient to keep the cells alive for the duration of the sort. Cells should be filtered through nylon mesh (70 microns maximum) immediately prior to sorting to prevent nozzle clogs - we can provide sterile filters for this purpose.. If your cells are particularly susceptible to clumping (as are many adherent cells), sorting the cells in Ca/Mg-free buffers and adding 0.1 mM EDTA may reduce aggregate formation, which can clog the sort nozzle and impede sort performance (TurboSort high-speed sorting is particularly susceptible to nozzle disruptions caused by

cell clumps and debris). Cells should be delivered in 12 x 75 mm Falcon (Becton-Dickinson) polystyrene or polypropylene tubes with no more than three milliliters volume per tube. We also highly recommend that you *count your cells immediately prior to sorting*. This will allow us to estimate efficiency of yield and to trace problems associated with low yield.

- Sorted cells are collected in 12 x 75 Falcon **polypropylene** (***not polystyrene***) tubes with the interiors coated with 0.5 mls 100% FBS. Polypropylene is less charged than polystyrene, reducing tight adherence of the sorted cells to the walls of the tube. The FBS reduces cell adherence to the walls of the tube, provides a "cushion" for the cells when they "hit", and keeps the cells happy for the duration of the sort. A sufficient number of these tubes should be prepared beforehand and delivered with the cells to be sorted.

- The FACSVantage SE is equipped with a temperature-controlled sample chamber and collection tube holder to keep both pre- and post-sorted cells at 4C or 37C. Let us know in advance if you require this system. Please supply an ice bucket for sample storage during and after the sort.

- Please try to start more or less on time (your experiment may not be the last one of the day!). If you are unavoidably delayed, contact us as soon as possible to inform us of the problem.

Protocol for sterile cell sorting.

The requirements for sterile cell sorting are similar to those for non-sterile sorting with the following modifications...

- Due to lab space limitations, we do not provide sterile sheath buffer. Please deliver 5 liters of sterile sheath buffer to the flow lab the day before the scheduled sort (2 liters are for presort instrument washing).

- On the day prior to the sort, the instrument is completely flushed with 10% bleach, followed by 70% EtOH. The instrument is then shut down with 70% EtOH still in the lines, and allowed to sit overnight. On the morning of the sort, the instrument is flushed with sterile sheath buffer for one hour prior to sorting. The interior of the sheath tank is also sterilized with 70% EtOH overnight prior to sorting. Immediately prior to the sort, a disposable sterile 0.2 um high-pressure fluid filter (Spectrum Mediacap) is installed on the sheath tank on the inlet line, providing an additional barrier to contamination. All surfaces of the instrument surrounding the sample port and collection tubes are swabbed with 70% EtOH prior to sorting.

- Tube changes (both sample and collection) provide opportunities for sterile sort contamination. Please take special care in filtering samples

prior to sorting, to prevent clogs and subsequent removal of the sample tube.

- Due to its complexity, cell sorting is generally considered to be an aseptic process (as opposed to truly sterile). Therefore, cells should be subsequently cultured with antibiotics (at least pen-strep) to prevent bacterial contamination.

If you have any questions regarding multilaser analysis or cell sorting, please don't hesitate to contact Bill Telford or Veena Kapoor for more information.

This material was prepared by the Telford Lab for the NCI ETI Branch and its friends. 10-16-99